Identification of *scyllo*-Inositol Phosphates in Soil by Solution Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy

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ABSTRACT

A large proportion of the organic P in soils can occur as *scyllo*-inositol phosphates. These compounds are rarely detected elsewhere in nature and remain poorly understood, partly because conventional procedures for their determination are lengthy and erroneous. We report a straightforward procedure for the determination of *scyllo*-inositol phosphates in soil extracts using solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy. Solution ^{31}P NMR chemical shifts of a range of synthetic *scyllo*-inositol phosphate esters were determined in alkaline solution. Of these, only the signal corresponding to *scyllo*-inositol hexa*kis*phosphate at approximately 4.2 ppm was identified in soil NaOH–EDTA extracts, constituting between 6.5 and 9.8% of the NaOH–EDTA extracted P. This signal has been previously assigned to choline phosphate, but we confirmed it to be an inositol phosphate using hypobromite oxidation, a procedure that destroys all organic matter except inositol phosphates. Lower order *scyllo*-inositol phosphate esters were not identified in the extracts studied here, and literature reports suggest that they probably occur in insufficient concentrations to be detected by this procedure. The identification of *scyllo*-inositol hexa*kis*phosphate in soils and other environmental samples will allow its quantification in a range of environments, and facilitate research into the origins and function of this enigmatic compound.

Detailed information on soil organic P is fundamental to understanding biogeochemical cycles in both natural and managed ecosystems (Condron et al., 2004). One of the most intriguing aspects of soil organic P is the presence of inositol phosphates that seldom occur elsewhere in nature. The most common stereoisomeric forms are *myo*-inositol phosphate, which originate mainly in plant seeds, but *scyllo*-, D-*chiro*-, and *neo*-inositol phosphates also occur in varying proportions (Cosgrove, 1980). In particular, a large proportion of the soil organic P can occur as *scyllo*-inositol phosphates, yet the origin and function of this enigmatic group of compounds remain unknown (Turner et al., 2002). Free *scyllo*-inositol has often been detected in animals and plants, but rarely in phosphorylated forms (Posternak, 1965). Indeed, the report of a *scyllo*-inositol phospholipid in barley (*Hordeum vulgare* L. cv Himalaya) seed was the first of a phosphorylated *scyllo*-inositol in any biological tissue (Narasimhan et al., 1997).

Investigation of *scyllo*-inositol phosphates in soils is limited by a lack of suitable analytical techniques for their determination, because conventional procedures involve lengthy extraction, clean up, and chromatographic separation steps. Soil organic P can be characterized by alkaline extraction and solution ^{31}P NMR spectroscopy (Condron et al., 1997), a procedure that has been improved recently by the adoption of a single-step NaOH–EDTA extraction, more accurate signal identification, and greater understanding of compound degradation during extraction and analysis (Cade-Menun and Preston, 1996; Makarov et al., 2002; Turner et al., 2003a). However, its use is limited for analysis of inositol phosphates due to poor resolution in the orthophosphate monoester region of the spectrum, although *myo*-inositol hexa*kis*phosphate, the most abundant component of soil organic P, can now be accurately quantified in complex spectra with the aid of simple deconvolution software (Turner et al., 2003c).

The aim of this study was to develop a procedure for the determination of *scyllo*-inositol phosphates in soil extracts using alkaline extraction and solution ^{31}P NMR spectroscopy. To achieve this we measured ^{31}P NMR chemical shifts of synthetic *scyllo*-inositol phosphate standards in alkaline solution, then used hypobromite oxidation to demonstrate the presence of *scyllo*-inositol hexa*kis*phosphate in alkaline extracts of soils.

MATERIALS AND METHODS

Solution ^{31}P NMR Spectroscopy of Standard *scyllo*-Inositol Phosphates

All *scyllo*-inositol phosphate standards were synthesized and purified by the late Dr. Dennis Cosgrove, formerly of CSIRO Plant Industry, Canberra, Australia. No formal record of the procedures used to prepare these compounds exists, but probable procedures based on Cosgrove's published methodologies are reported below.

The following samples were analyzed: a *scyllo*-inositol hexa*kis*phosphate prepared by phosphorylation of *scyllo*-inositol by heating with polyphosphoric acid, and purified from lower esters by ion-exchange chromatography (Cosgrove, 1966); a *scyllo*-inositol hexa*kis*phosphate extracted from soil organic matter and presumably isolated by alkaline extraction and ion-exchange chromatography (Cosgrove, 1963); a *scyllo*-inositol penta*kis*phosphate and two *scyllo*-inositol tetra*kis*phosphate esters prepared by hydrolysis of *scyllo*-inositol hexa*kis*phosphate by a wheat-bran phytase (Lim and Tate, 1973); a *scyllo*-inositol *tris*phosphate and a *scyllo*-inositol *bis*phosphate prepared using phytase isolated from a soil *Pseudomonas* (Irving and Cosgrove, 1971). All compounds were prepared as Ba salts, except the soil-derived *scyllo*-inositol hexa*kis*phosphate, which was a free acid.

The *scyllo*-inositol phosphate standards were prepared for solution ^{31}P NMR spectroscopy by mixing 10 mg of the Ba salt with 5 mL of deionized water and 5 mL of Amberlite IR-120 (H^+) cation exchange resin (Sigma-Aldrich Co., St. Louis, MO). The resin was prepared initially by washing sequentially

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Table 1. Properties of the three soils used in the current study.

	Soil 1	Soil 2	Soil 3
UK soil series	Denbigh	Brickfield	Fladbury
USDA class	Dystrochrepts	Haplaquepts	Fluvaquents
Topsoil texture	Clay	Clay loam	Clay
Location	Camelford, Cornwall	Llangefni, Anglesey	Glastonbury, Somerset
Total C, g kg^{-1} soil	58.7	46.0	80.4
Total N, g kg^{-1} soil	6.35	4.83	8.70
Total P, g kg^{-1} soil	1.54	1.11	1.98
Clay (<2 μm), g kg^{-1} soil	445	335	681
Soil pH (water)	4.5	4.8	5.0
Oxalate Fe, g kg^{-1} soil	7.7	5.3	12.9
myo-inositol hexa*kis*phosphate, mg P kg^{-1} soil†	189	144	163

† Determined in NaOH–EDTA extracts by solution ^{31}P NMR spectroscopy and spectral deconvolution (Turner et al., 2003c).

in deionized water, 1 *M* HCl, then again in deionized water. After swirling the slurry for several minutes, the mixture was filtered through a 0.2-μm cellulose-acetate syringe filter (Nalgene, Rochester, NY) and the resin washed three times with 5-mL aliquots of deionized water. The filtrate and washings were combined, made alkaline with a few drops of 1 *M* NaOH, and then evaporated to dryness at 45°C.

For solution ^{31}P NMR spectroscopy, each dried residue was redissolved in 0.8 mL of 1 *M* NaOH, 0.1 mL of 10 mg L^{-1} K_2HPO_4, and 0.1 mL of D_2O, and transferred to a 5-mm NMR tube. The NaOH ensured a pH > 13 (for consistent chemical shifts and optimum spectral resolution), the K_2HPO_4 provided a reference signal, and the D_2O provided an NMR signal lock. Solution ^{31}P NMR spectra were obtained using a Bruker Avance DRX 500 MHz spectrometer operating at 202.456 MHz for ^{31}P and 500.134 MHz for ^{1}H. Temperature was regulated at 27°C, and broadband proton decoupling was used for all samples. Samples were analyzed using a 5-μs pulse (45°), a delay time of 20.0 s, and an acquisition time of 0.8 s. The relatively long delay time ensured sufficient spin-lattice relaxation between scans for P nuclei in these samples. Approximately 150 scans were acquired for each spectrum, representing <1 h of machine time, and spectra were plotted using a line broadening of 0.3 Hz. Chemical shifts of signals were determined in parts per million (ppm) relative to an external standard of 85% H_3PO_4 (w/w).

Soil Extraction and Analysis

Three lowland permanent pasture soils from the UK were extracted to investigate possible signals from *scyllo*-inositol phosphates. Soil physical and chemical properties are reported in Table 1. Recent studies have determined the P composition of these soils by solution ^{31}P NMR spectroscopy (Turner et al., 2003b), and the concentrations of *myo*-inositol hexa*kis*phosphate using a novel spectral deconvolution procedure (Turner et al., 2003c).

Phosphorus was extracted by shaking 5 g of soil with 100 mL of a solution containing 0.25 M NaOH and 0.05 M EDTA for 16 h at 20°C (Cade-Menun and Preston, 1996). The extracts were centrifuged at 10 000 × *g* for 30 min and half of each extract immediately frozen at −80°C. The remaining half was treated with hypobromite oxidation based on methodology described in Irving and Cosgrove (1981) and Suzumura and Kamatani (1993). This technique oxidizes all organic matter except inositol phosphates. Briefly, 50 mL of extract was placed in a boiling tube, cooled in an ice bath, and 2 mL of pure Br (also cooled in an ice bath) added slowly in 0.5-mL aliquots. The mixture was left at room temperature for 1.5 h to allow oxidation to proceed, then boiled for 5 min to remove volatile reaction products. After cooling, 2 mL of concentrated NH_4OH was added to convert hypobromite to Br_2, and then the solution was acidified to pH < 3 with 10 *M* HCl to destroy carbonates. A small amount of concentrated NH_4OH was added to convert Br to Br_2, and the pH adjusted to >10 with 5 *M* NaOH. The solution was then frozen at −80°C.

The brominated and untreated extracts were lyophilized, and the residues ground to a fine powder. For solution ^{31}P NMR spectroscopy, each freeze-dried extract (approximately 100 mg) was redissolved in 0.9 mL of 1 *M* NaOH and 0.1 mL of D_2O, and transferred to a 5-mm NMR tube. Brominated extracts were also analyzed by redissolving in 0.9 mL of a solution containing 1 *M* NaOH and 0.1 *M* EDTA (and 0.1 mL D_2O), which resulted in markedly improved resolution (see *Results* below). The pH of the redissolved samples varied slightly, but was always >13. Machine parameters were identical to those used for analysis of *scyllo*-inositol phosphate standards, except for a delay time of 1.0 s. This shorter delay time could be used because the extracts contained large concentrations of paramagnetic ions, which shorten spin-lattice relaxation times by helping P nuclei to relax more rapidly (Cade-Menun et al., 2002).

Chemical shifts were assigned to individual P compounds or functional groups based on literature reports (Turner et al., 2003a), with signal areas calculated by integration. Spectra were plotted using a 5-Hz line broadening, although additional spectra of brominated samples redissolved in NaOH plus EDTA were also plotted using a 1-Hz line broadening to conserve enhanced resolution.

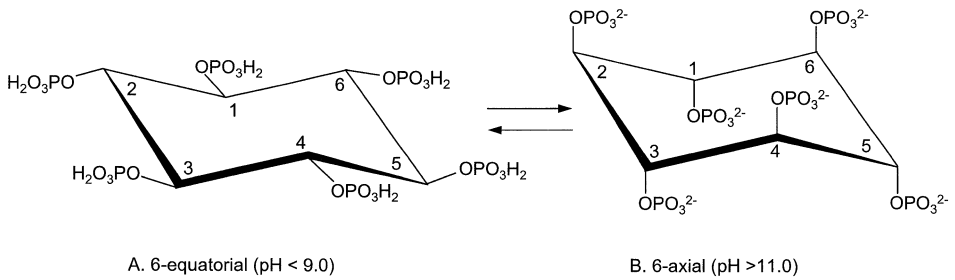

Fig. 1. Structure of *scyllo*-inositol hexa*kis*phosphate in solution: (a) the all equatorial structure at pH < 9.0, (b) the all axial structure at pH > 11.0 (Volkmann et al., 2002).

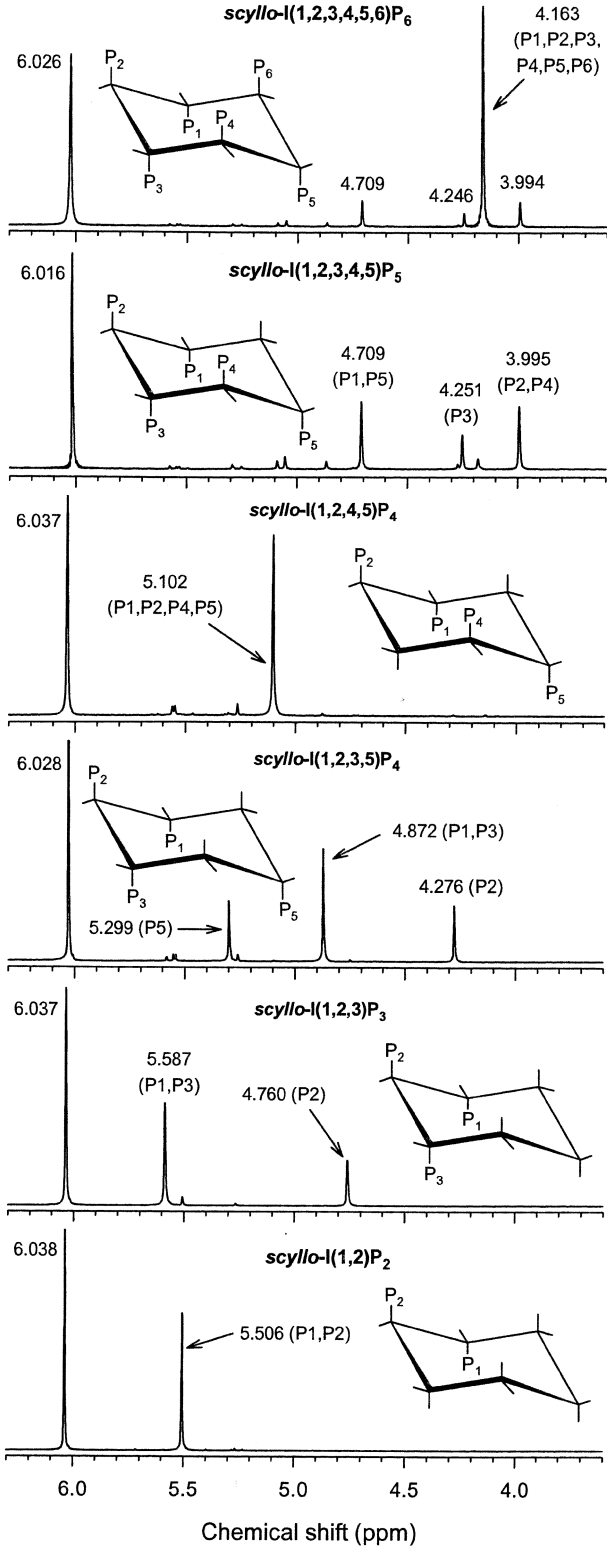

Fig. 2. Spectra of *scyllo*-inositol phosphate standards synthesized by the late Dr. Dennis Cosgrove determined by solution ^{31}P NMR spectroscopy. Compounds were analyzed at pH > 13. Phosphate groups responsible for the individual signals are indicated by the position of the phosphate on the inositol ring.

RESULTS

Conformation of *scyllo*-Inositol Hexa*kis*phosphate at pH > 13

Of the nine possible inositol stereoisomers, *scyllo*-inositol is the most simple, because all hydroxyl groups are structurally equivalent, being either all axial or all equatorial depending on solution pH (Fig. 1). Thus, *scyllo*-inositol differs from the *myo* isomer by the orientation of only a single hydroxyl group. It was reported that *scyllo*-inositol hexa*kis*phosphate displays an equatorial structure at pH < 9 and an all-axial structure at pH > 11; between these pH values there is probably dynamic equilibrium between the two conformations (Volkmann et al., 2002). The pH of the samples analyzed by solution ^{31}P NMR spectroscopy in our experiments was always >13, so *scyllo*-inositol hexa*kis*phosphate was in the all-axial conformation. Based on evidence of the conformation of lower *myo*-inositol phosphate esters at different pH values (Barrientos and Murthy, 1996), it seems likely that the lower *scyllo*-inositol phosphate esters display similar conformational changes to *scyllo*-inositol hexa*kis*phosphate at different pH values, although there is no direct evidence for this.

Solution ^{31}P NMR Spectroscopy of *scyllo*-Inositol Phosphates

Solution ^{31}P NMR spectra of *scyllo*-inositol phosphate standards are shown in Fig. 2. Details of contributing P groups and chemical shifts standardized to that of orthophosphate at 6.0 ppm are also reported (Table 2). Signals for *scyllo*-inositol phosphate regioisomers not analyzed here were calculated from data for synthetic compounds reported in Chung et al. (1999) (Table 2).

The synthetic *scyllo*-inositol hexa*kis*phosphate gave a single signal at 4.163 ppm (Fig. 2), and a similar signal at 4.155 ppm was detected for the same compound extracted from soil organic matter (not shown). This single signal arises because the chemical environments around all six phosphate moieties in *scyllo*-inositol hexa*kis*phosphate are identical. In both spectra, small signals at approximately 3.99, 4.24, and 4.70 ppm indicated the presence of trace amounts of *scyllo*-inositol penta*kis*-phosphate (see below).

There is only one form of *scyllo*-inositol penta*kis*phosphate, here termed *scyllo*-inositol (1,2,3,4,5) penta*kis*-phosphate for simplicity (labeling is arbitrary as all phosphates are structurally identical). Signals from this compound were detected at 3.994, 4.251, and 4.709 ppm in the ratio 2:1:2. A small signal at 4.179 ppm indicated a trace of *scyllo*-inositol hexa*kis*phosphate, while other small signals between 4.8 and 5.6 ppm indicated the presence of lower order *scyllo*-inositol phosphates.

The two *scyllo*-inositol tetra*kis*phosphate esters gave markedly different spectra. One gave a single signal at 5.102 ppm and was thus identified as *scyllo*-inositol (1,2,4,5) tetra*kis*phosphate (Chung et al., 1999). The other gave signals at 4.276, 4.872, and 5.299 ppm in the ratio 1:2:1, and was thus identified as *scyllo*-inositol (1,2,3,5) tetra*kis*phosphate (Chung et al., 1999).

Table 2. Solution ^{31}P NMR chemical shifts of all possible forms of *scyllo*-inositol phosphates in alkaline solution. Values were determined by spectroscopy of compounds purified by the late Dr. Dennis Cosgrove, or calculated from literature values (Chung et al., 1999).

Compound	Chemical shift†	Number of P groups	Contributing P groups	Reference‡
	ppm			
scyllo-I(1,2,3,4,5,6)P_6	4.137	6	P1, P2, P3, P4, P5, P6	This study
scyllo-I(1,2,3,4,5)P_5	3.979	2	P2, P4	This study
	4.235	1	P3	
	4.693	2	P1, P5	
scyllo-I(1,2,4,5)P_4	5.065	4	P1, P2, P4, P5	This study
scyllo-I(1,2,3,5)P_4	4.248	1	P2	This study
	4.844	2	P1, P3	
	5.271	1	P5	
scyllo-I(1,2,3,4)P_4	4.127	2	P2, P3	Chung et al. (1999)
	4.987	2	P1, P4	
scyllo-I(1,2,3)P_3	4.723	1	P2	This study
	5.550	2	P1, P3	
scyllo-I(1,2,4)P_3	5.147	1	P4	Chung et al. (1999)
	5.477	2	P1, P2	
scyllo-I(1,3,5)P_3	5.217	3	P1, P2, P5	Chung et al. (1999)
scyllo-I(1,2)P_2	5.468	2	P1, P2	This study
scyllo-I(1,3)P_2	5.277	2	P1, P3	Chung et al. (1999)
scyllo-I(1,4)P_2	5.157	2	P1, P4	Chung et al. (1999)

† Corrected for orthophosphate chemical shift at 6.0 ppm.
‡ Chemical shifts calculated from Chung et al. (1999) are standardized from values determined at pH 10 relative to that of *scyllo*-inositol hexa*kis*phosphate at 4.137 ppm.

The *scyllo*-inositol *tris*phosphate gave signals at 4.760 and 5.587 ppm in the ratio 1:2, and was thus identified as *scyllo*-inositol (1,2,3) *tris*phosphate (Chung et al., 1999). The *scyllo*-inositol *bis*phosphate gave a single signal at 5.506 ppm, which based on the signals for the three possible *bis*phosphate esters was identified as the *scyllo*-inositol (1,2) *bis*phosphate, rather than the (1,3) or (1,4) esters (Chung et al., 1999).

Identification of *scyllo*-Inositol Phosphates in Alkaline Soil Extracts

Solution ^{31}P NMR spectra of soil NaOH–EDTA extracts are shown in Fig. 3, with the proportions of individual compounds reported in Table 3. Brominated extracts redissolved in NaOH gave poorly resolved spectra, with significant line broadening that obscured all but the strongest signals (Fig. 3). However, when extracts were redissolved in NaOH plus EDTA, spectral resolution improved markedly, as demonstrated by the expanded spectra plotted with 1 Hz line broadening. It should be noted that chemical shifts in brominated extracts were slightly upfield of those in untreated extracts, probably due to greater salt concentrations in the brominated extracts. For example, orthophosphate appeared between 6.16 and 6.19 ppm in untreated extracts, but between 6.24 and 6.43 ppm in brominated extracts.

The strong signal at approximately 4.2 ppm in all extracts has been previously assigned to choline phosphate, because soil extracts spiked with this compound gave a signal at 4.05 ppm in alkaline solution (Turner et al., 2003a). However, the signal is coincident with that from *scyllo*-inositol hexa*kis*phosphate (Fig. 2), and remained unchanged in all three extracts following bromination (Table 3, Fig. 3). This confirms the assignment of this signal to *scyllo*-inositol hexa*kis*phosphate, because all organic phosphates except higher-order inositol phosphates are destroyed by hypobromite oxidation (Wrenshall and Dyer, 1941; Irving and Cosgrove, 1981; Nanny and Minear, 1997). This was further supported by corresponding increases in the proportion of orthophosphate measured in brominated extracts (Table 3).

Clear signals in the orthophosphate monoester region at approximately 5.9, 5.0, 4.7, and 4.5 ppm in the ratio 1:2:2:1 originated from *myo*-inositol hexa*kis*phosphate (Turner et al., 2003a). The contribution of this compound to the total spectral area was similar in untreated and brominated extracts (Table 3), demonstrating clearly that it was unaffected by bromination (Irving and Cosgrove, 1981).

Signals upfield of orthophosphate at approximately 6.6 and 6.8 ppm in all three untreated extracts have been previously assigned to compounds similar in structure to aromatic orthophosphate diesters (Bedrock et al., 1994; Turner et al., 2003a). However, these signals remained relatively unchanged by bromination, indicating that they represent unidentified inositol phosphates. More detailed studies are required to unambiguously identify these signals, although a sample of mixed *neo*-inositol penta*kis*phosphates gave similar signals in this region of the spectrum (data not shown).

Signals from DNA between −0.12 and −0.23 ppm in untreated extracts were hardly detectable in brominated extracts, indicating that DNA was at least partly degraded by the bromination procedure. This is in contrast to a previous literature report (Nanny and Minear, 1997). However, pyrophosphate signals at −4.3 ppm were relatively unaltered by bromination, suggesting that simple P speciation by molybdate colorimetry cannot be used to determine the inositol phosphate fraction in brominated extracts.

DISCUSSION

The identification of the strong signal at approximately 4.2 ppm in alkaline soil extracts as *scyllo*-inositol hexa*kis*phosphate is significant, because this signal is prominent in solution ^{31}P NMR spectra of extracts of

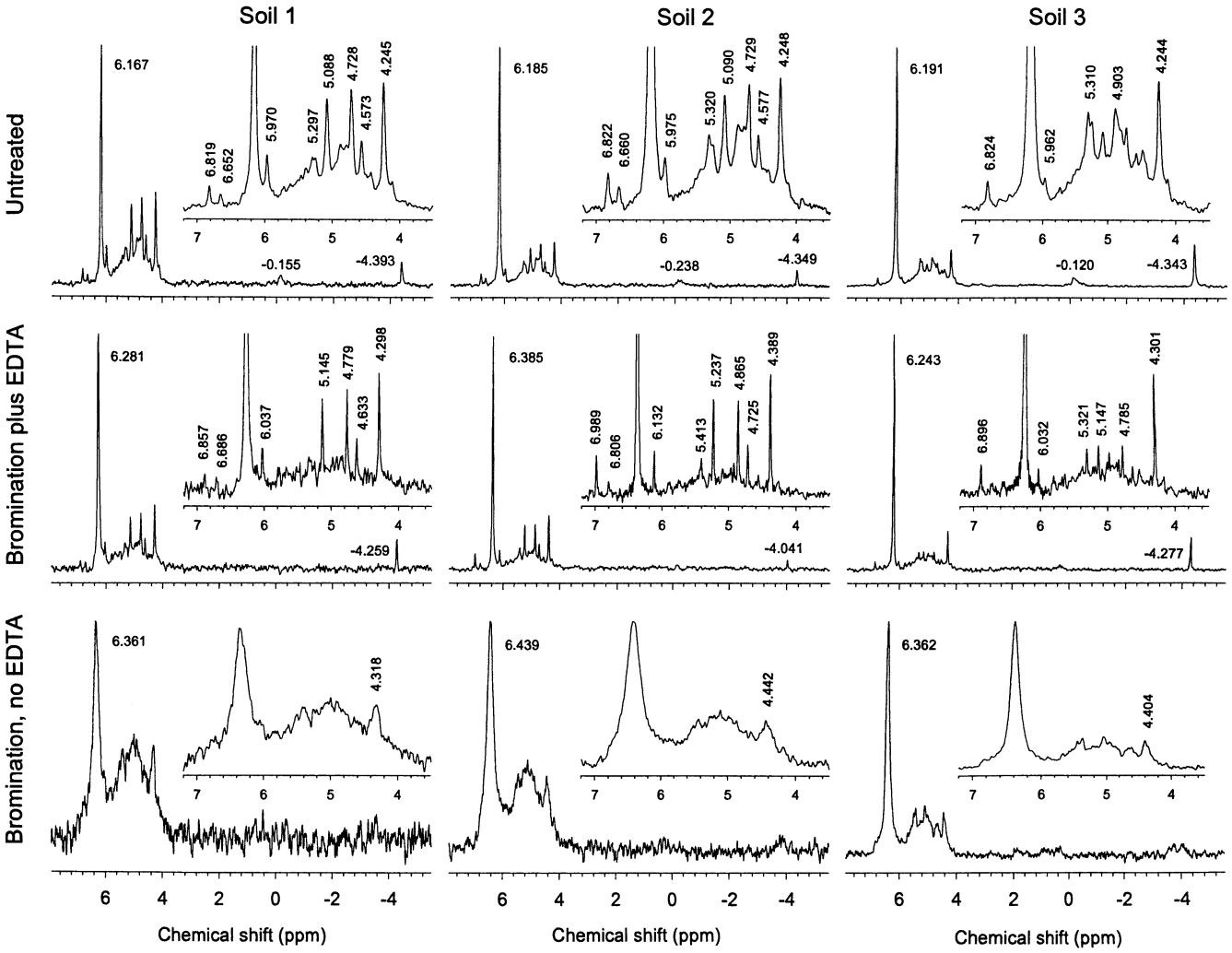

Fig. 3. Solution ^{31}P NMR spectra of NaOH–EDTA extracts of three temperate pasture soils from England and Wales. Extracts were analyzed untreated and after pretreatment by hypobromite oxidation to destroy all organic matter except the inositol phosphates. Brominated samples were analyzed after redissolving in either 1.0 *M* NaOH or 1.0 *M* NaOH plus 0.1 *M* EDTA. Chemical shifts are shown in ppm relative to an external H_3PO_4 standard. All spectra are plotted using a 5-Hz line broadening, except the inset spectra of the brominated extracts redissolved in NaOH plus EDTA, which are plotted with 1-Hz line broadening to preserve enhanced spectral resolution.

many different types of soils reported in the literature. It is impossible to rule out the contribution of choline phosphate to this signal without bromination, although the rapid turnover of labile orthophosphate monoesters in soil (Bowman and Cole, 1978) suggests that choline phosphate is unlikely to be quantitatively important in most cases. It is also unlikely to originate from the breakdown of phosphatidyl choline during extraction and analysis, because this leads to β-glycerophosphate (4.81 ppm) and phosphatidic acid (5.15 ppm) rather than choline phosphate (Turner et al., 2003a). Further, signals from choline phosphate and *scyllo*-inositol hexa*kis*phosphate are not exactly coincident,, occurring at 4.02 and 4.14 ppm, respectively, when corrected for the

Table 3. Proportions of P compounds determined by alkaline extraction and solution ^{31}P NMR spectroscopy of three temperate pasture soils. Extracts were analyzed without pretreatment or following hypobromite oxidation to destroy all organic matter except inositol phosphates.

		Soil 1		Soil 2		Soil 3	
	Chemical shift†	Untreated	Brominated	Untreated	Brominated	Untreated	Brominated
	ppm	% NaOH–EDTA extractable P					
myo-inositol hexa*kis*phosphate‡	4.5, 4.7, 5.0, 5.9	18.9	18.9	15.8	16.2	7.5	7.8
scyllo-inositol hexa*kis*phosphate	4.2	8.9	8.8	8.0	9.8	6.6	6.5
Orthophosphate	6.2	22.7	65.7	36.4	55.1	38.9	57.4
Pyrophosphate	−4.3	2.6	6.7	2.3	1.4	8.5	8.8
DNA	−0.12 to −0.24	3.0	ND	3.0	ND	4.9	ND

ND, not detected.
† Based on approximate chemical shift values (Fig. 3).
‡ Values calculated by multiplying by six the signal from the phosphate at the C2 position on the inositol ring at approximately 5.9 ppm (Turner, 2004).

chemical shift of orthophosphate at 6.0 ppm. It therefore seems certain that *scyllo*-inositol hexa*kis*phosphate represents a significant component of soil organic P (6–10% NaOH–EDTA extractable P in the three soils analyzed here), confirming previous studies that have identified *scyllo*-inositol phosphates in soils using conventional chromatography (Cosgrove, 1980; reviewed recently by Turner et al., 2002).

Identification and quantification of *scyllo*-inositol hexa*kis*phosphate in soil extracts using solution ^{31}P NMR spectroscopy is simplified by the strength of the signal, which arises because the environments around all six phosphates are chemically identical. This is in contrast to the four signals from *myo*-inositol hexa*kis*-phosphate in the ratio 1:2:2:1, although it is now possible to quantify these relatively easily using spectral deconvolution software (Turner et al., 2003c). Chemical shifts of lower order *scyllo*-inositol phosphate esters are reported, but were not identified in the soils analyzed here. The presence of *scyllo*-inositol penta*kis*phosphate would be most readily detected by the signal at 3.99 ppm from the C2 and C4 phosphates (Fig. 2), because this would be well separated from the main envelope of signals in the orthophosphate monoester region. However, based on literature information it seems unlikely that the penta*kis*phosphates would occur in sufficient quantities to permit detection by solution ^{31}P NMR spectroscopy.

In poorly resolved spectra, pretreatment of samples by hypobromite oxidation will markedly improve the accuracy of *scyllo*-inositol hexa*kis*phosphate quantification, and eliminate the possible contribution of choline phosphate. The technique also markedly improves spectral resolution, providing that extracts are redissolved in NaOH plus EDTA, rather than NaOH alone. The poor spectral resolution of brominated extracts redissolved in NaOH alone is probably due to destruction of EDTA during hypobromite oxidation, which allows P nuclei to come into close proximity with paramagnetic ions. Reintroducing EDTA prevents this interaction by chelating paramagnetic ions, yet maintains them in solution to allow short delay times to be used. The inclusion of EDTA in the NMR tube is therefore likely to be important when analyzing other types of samples that contain interfering paramagnetic ions, such as water or organic anion extracts of soils.

The ability to quantify *scyllo*-inositol hexa*kis*phosphate in soil extracts will facilitate more detailed and widespread investigation of this intriguing organic phosphate. The origins of *scyllo*-inositol hexa*kis*phosphate in soils remain unclear, but the fact that it has been detected only in soils and aerobically digested sewage sludge suggests a probable microbial source (Cosgrove, 1980). Indeed, an isomer of *myo*-inositol hexa*kis*phosphate, subsequently shown by Cosgrove to be *scyllo*-inositol hexa*kis*phosphate, was detected after a sand-clay mixture containing inorganic and organic nutrients was incubated with soil microorganisms (Caldwell and Black, 1958). However, it remains unclear whether microbes directly synthesize *scyllo*-inositol hexa*kis*phosphate from carbohydrate precursors, or epimerize it from *myo*-inositol hexa*kis*phosphate. Whatever the mechanism involved, the ecological function of *scyllo*-inositol hexa*kis*phosphate in soils is completely unknown. Future research should focus on quantifying the concentrations of *scyllo*-inositol hexa*kis*phosphate in soils from a wide range of environments, and investigating its biochemical origin and function in soil.

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